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Serum Adenosine Deaminase (ADA) Activity: A Novel Screening Test to Differentiate HIV Monoinfection From HIV-HBV and HIV-HCV Coinfections

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Background: $CD4^+$ cell count, the common HIV infection screening test, is costly and unable to differentiate HIV monoinfection from its concurrent infection with hepatitis B or C virus. We aimed to ascertain diagnostic value of serum adenosine deaminase (ADA) activity as a useful tool to differentiate HIV mono- and co-infection. *Methods:* Blood samples were collected from 30 HIV-HBV and 30 HIV-HCV coinfected patients, 33 HIV positive subjects, and 72 controls. $CD4^+$ cell count, serum total ADA (tADA), and ADA1, and ADA2 isoenzyme activities were determined and their sensitivity and specificity were computed. *Results:* tADA and ADA2 activities were significantly higher and $CD4^+$ counts were markedly lower in all patients compared with controls. Strong inverse agreements between $CD4^+$ cell counts and both tADA and ADA2 activities were observed. Serum tADA and ADA1 activities showed the highest specificity and the highest sensitivity, respectively, for differentiating HIV monoinfection from HIV-HBV and HIV-HCV coinfections. *Conclusions:* We showed strong agreement and correlation between $CD4^+$ cell count and ADA enzyme activity. Based on high ADA sensitivity and specificity, it is concluded that determination of ADA activity might be a novel diagnostic tool to distinguish of HIV monoinfection from its coinfection with HBV or HCV. J. Clin. Lab. Anal. 30:200–203, 2016.

Key words: adenosine deaminase; coinfection; diagnosis; human immunodeficiency virus; hepatitis B virus; hepatitis C virus

In western countries it is estimated that 25-30% of people living with human immunodeficiency virus (HIV) are coinfected with hepatitis C (HCV) and 6-14% with hepatitis B viruses (1). HIV-HBV and HIV-HCV coinfection has attracted much attention since morbidity and mortality in HIV–infected individuals in regions where injection drug users represent a large proportion of the HIV–positive population markedly increased (2), especially in those with low $CD4^+$ cell counts (3–5). Early diagnosis

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The Author name Khatooni was spelled out incorrectly. Now it was changed from Khatoni to Khatooni on 10th August 2015 after first online publication date.

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of both HIV mono– and co–infection with HCV or HBV is essential to most effectively manage infected individuals. However, viral loads and $CD4^+$ counts are costly and require highly skilled laboratory personnel and complicated equipments (6). Additionally, HIV coinfected patients should also be diagnosed and differentiated from simple HIV infected persons by evaluating hepatitis B or C viral load which is not usually applicable in resource–restricted areas.

Serum total ADA activity or its ADA1 and ADA2 isoenzyme activities have frequently been used as diagnostic biomarkers in screening of infectious diseases (7–9). Similarly, we have recently reported a significant increase in ADA activity in HIV–HBV coinfection confirming usefulness of ADA activity as a simple, rapid, and inexpensive diagnostic marker for screening and monitoring of HIV positive and HIV-HBV coinfected patients compared with other costly, laborious, and time–consuming diagnostic tools (10). This study was aimed to ascertain the reliability of determination of serum ADA activity to differentiate HIV infected patients from healthy subjects and HIV monoinfected patients from HIV–HCV and HIV-HBV coinfected individuals.

Subjects including 33 HIV monoinfected, 30 HIV-HBV and 30 HIV-HCV coinfected patients, and 72 controls were enrolled in the study. Written informed consent for participation was obtained and the project was approved by the Research Ethics Committee of Kurdistan University of Medical Sciences (Iran). Subjects with blood $CD4^+$ cell counts lower than 200 cells/μL or those with more than 3 years of HIV infection history, subjects under antiretroviral therapy, and patients with a history of alcohol abuse, diabetes mellitus, tuberculosis, and cardiac or renal failure were excluded from the study.

Fasting blood samples were used for $CD4^+$ cell count and serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) activities were determined, as markers of liver injury. HIV positive results were confirmed by Western Blotting (11). HBV infection was detected by HBs antigen test and reconfirmed by recombinant immunoblot test, whereas HCV infection was detected by anti–HCV antibody ELISA kits and reconfirmed by HCV recombinant immunoblot assay (RIBA) (12). Serum total ADA (tADA) and its ADA1 and ADA2 isoenzyme activities were measured according to Giusti method (13).

Patients (mono- and coinfected) had significantly lower mean $CD4^+$ cell counts than controls and statistical analysis showed a trend to lower levels of cell counts in coinfected patients compared with HIV monoinfected individuals. However, $CD4^+$ cell counts did not significantly differ in HIV mono- and co-infection and no difference was found in $CD4^+$ count between HIV-HBV and HIV-HCV coinfected patients (Table 1).

TABLE 1. Personal Characteristics, Activities of Liver Enzymes, $Cd4^+$ Cell Counts, and Activities of Adenosine Deaminases in Healthy Subjects and Infected Patients

Subjects	N	Gender M/F	Age-Year Mean (Range)	AST (U/L) Mean (SD)	ALT (U/L) Mean (SD)	ALP (U/L) Mean (SD)	$CD4^+$ count (Cells/μL) Mean (SD)	tADA (U/L) Mean (SD)	ADA1 (U/L) Mean (SD)	ADA2 (U/L) Mean (SD)
Control	72	71/1	35 (25–60)	27.2 (4.0)^{a,b,f}	38.2 (5.4)^{a,b,c}	85.1 (3.6)	1748.3 (354.3)^{a,b,c,d}	23.4 (11.0)^{a,b,d,e}	6.3 (3.9)^{a}	16.4 (9.7)^{a,b,d,e}
HIV	33	28/5	36 (30–55)	36.0 (2.9)^{a,c,d}	48.1 (3.6)^{a,d,e,g}	86.9 (6.1)	522.8 (275.4)^{a}	**51.5 (12.5)^{a,c,f,g}**	8.1 (5.3)	**43.4 (12.5)^{a,c,f,g}**
All coinfections	60	57/3	34 (23–55)	34.1 (9.1)^{f}	43.1 (9.1)^{g}	85.7 (4.3)	445.1 (218.5)^{b}	60.8 (12.2)^{b,c}	8.2 (5.2)	52.7 (12.8)^{b,c}
HIV-HBV	30	30/0	34 (25–54)	42.5 (4.2)^{b,c,e}	51.3 (3.8)^{b,d,f}	86.7 (9.4)	387.7 (181.5)^{c}	†**59.7 (12.5)^{d,f}**	8.4 (5.4)^{a}	†**51.4 (13.2)^{d,f}**
HIV-HCV	30	27/3	35 (23–55)	26.4 (4.0)^{d,e}	35.5 (4.7)^{c,e,f}	84.8 (4.0)	517.7 (249.5)^{d}	‡**58.7 (12.7)^{e,g}**	7.7 (4.9)	‡**50.9 (15.3)^{e,g}**

SD, standard deviation; data represented as Mean (SD).
F, female; M, male; U/L, units per liter of enzyme activity.
In each column, data with similar superscript letters (a–g) represents significant difference ($P < 0.05$) between groups
[a] and [†] represent significant difference of given coinfected group compared with HIV monoinfected subjects with a P value of $P = 0.01$ and $P = 0.02$, respectively.

All mono- and co-infected patients had higher levels of serum tADA activity than controls. Coinfected individuals showed a greater enhancement in mean tADA activity than monoinfected patients. Mean ADA2 activity showed a nearly threefold increase in patients (all mono– and co–infected) compared with controls and was significantly higher in coinfected subjects than HIV monoinfected patients. Linear regression analysis confirmed that serum total ADA activity inversely increased ($R^2 = 0.625$, $P < 0.001$) by decreasing of $CD4^+$ cell counts. Similar inverse correlation ($R^2 = 0.587$, $P < 0.001$) was also observed between serum ADA2 isoenzyme activity and $CD4^+$ cell counts. Regression analysis also showed that tADA activity directly correlated to the ADA2 isoenzyme activity ($R^2 = 0.945$, $P < 0.05$). According to R software statistics, serum enzymatic activities of tADA, ADA1, and ADA2 significantly ($P < 0.001$) differed in subjects with different $CD4^+$ cell counts and those with highest cell counts (controls) showed the lowest adenosine deaminase activity whereas the highest enzyme activity was found in patients with lowest cell counts (data is not shown).

Based on receiver operating characteristic (ROC) analysis, cutoff scores for $CD4^+$ cell count, tADA, ADA1, and ADA2 enzyme activity were defined as 1067 cells/μl, 40 U/L, 6.62 U/L, and 29.38 U/L, respectively, to discriminate healthy subjects from patients (all mono- and co-infected). Similarly, to differentiate HIV monoinfected subjects from coinfected patients (HIV-HBV and HIV-HCV), cutoff values were defined as 846 cells/μl, 58.33 U/L, 4.95 U/L, and 47.73 U/L for $CD4^+$ cell count, tADA, ADA1, and ADA2 enzyme activity. ROC analysis for HIV monoinfection against HIV coinfection showed significant ($P < 0.001$) sensitivity, specificity, and area under curve for both tADA and ADA2 activities. The highest specificity (82%) was observed for tADA activity whereas ROC analysis showed the greatest sensitivity (80%) for ADA1 enzyme activity in differentiating HIV mono- and coinfected patients. Moreover, Kappa coefficients of agreement between $CD4^+$ cell count and tADA, ADA1, and ADA2 enzyme activities were calculated as 0.815, 0.164, and 0.824, respectively, and showed strong agreements between $CD4^+$ cell counts and both tADA and ADA2 activities corresponding to significantly high areas under the curve (0.902 and 0.899, respectively).

In the present study, we assessed the diagnostic validity of tADA and its isoenzymes activities in HIV mono- and coinfected patients compared to healthy subjects. We showed a significant decline of $CD4^+$ cell counts in HIV positive patients, as previously been described (14) indicating that HIV infection is primarily targeted against $CD4^+$ cells (14,15). Our results also confirmed that a great $CD4^+$ cell depletion occurred in individuals with HIV-HBV and HIV-HCV coinfection, an observation that is in line with previous studies and indicates consistency of $CD4^+$ cell reduction with different immunodeficiency diseases (16–18). However, the present study showed no significant difference in $CD4^+$ cell count between HIV mono- and co-infected patients, and $CD4^+$ cell count was not an appropriate tool to differentiate HIV positive patients from HIV-HBV and HIV-HCV coinfected subjects. To overcome this problem, we evaluated serum activity of adenosine deaminase and its isoenzymes, ADA1 and ADA2, as effective surrogate markers for $CD4^+$ cell count.

A higher activity of ADA was detected in all HIV mono- and co-infected patients compared with controls. Our observation was in line with previous reports indicating that HIV infection alters serum ADA activity (8, 19), therefore, elevated plasma ADA activity might be considered as a useful surrogate marker for HIV infection that occurs early in the disease process. The increasing of serum tADA activity in co-infected patients was significantly greater than those of HIV mono-infected individuals. This observation is supported by our previous report confirming a greater rise in tADA activity in HIV-HBV co-infection compared with HIV monoinfection (10). Here, we confirmed that HIV-HCV coinfection can also enhances tADA activity greater than HIV monoinfection. Based on these evidences, serum tADA activity can now be introduced as a novel differential marker for HIV infection and its coinfection with hepatitis B or C viruses.

Additionally, serum tADA activity increased with decreasing of $CD4^+$ cell counts in all subjects. A similar inverse correlation was also observed between ADA2 isoenzyme activity and $CD4^+$ cell count. These results are supported by previous findings revealing that plasma ADA activity, including ADA1 and ADA2 isoenzymes, has negative correlation with $CD4^+$ cell counts (10, 15) and suggest that plasma ADA can be a sensitive marker of an ongoing biological insult to host immune system. Increasing of tADA activity might be due to increase in ADA2 isoenzyme activity, as a strong direct correlation was observed between tADA and ADA2 activities in this study.

In line with previous reports, our results showed different enzymatic activities of tADA, ADA1, and ADA2 in subjects with different $CD4^+$ cell counts and the highest adenosine deaminase activity was observed in subjects with the lowest $CD4^+$ cell counts representing later stage of disease (14). In contrast, the least tADA and ADA2 activities there existed in the subjects with the highest $CD4^+$ cell counts (controls). Therefore, the gradual increasing of enzyme activity in accordance to the stepwise decreasing of $CD4^+$ cell counts indicates that tADA activity increases by the worsening of disease. It can be concluded that as the reduction in $CD4^+$ cell counts represents the stages of disease (14), increasing of tADA activity might also be used to indicate progression of disease.

As in our recent work, we found a high sensitivity and specificity for both tADA (88% and 96%, respectively) and ADA2 (93% and 90%, respectively) activities confirming applicability of serum adenosine deaminase as a novel marker in distinguishing HIV positive patients (all mono- and co-infected) from healthy subjects (20). After redefining of cut-off values for $CD4^+$ cell count, tADA, ADA1, and ADA2 enzyme activity, ROC curve analysis for HIV monoinfection against HIV coinfections showed a significant sensitivity, specificity, and area under curve for all tADA, ADA1, and ADA2 enzyme activities with the highest specificity (82%) being observed for tADA and the greatest sensitivity (80%) for ADA1. Moreover, based on Kappa coefficients strong agreement between $CD4^+$ cell counts and both tADA and ADA2 activities provided further evidences in concurrent changes of $CD4^+$ cell count and ADA activities. Additionally, tADA showed the highest specificity whereas ADA1 showed the highest sensitivity for differentiating of HIV mono-infection from its coinfection by HBV or HCV. Therefore, it can be concluded that simultaneous determination of enzymatic activities of tADA and its isoenzymes, ADA1 and ADA2, might be a useful diagnostic tool in screening of aforementioned coinfected patients. To our knowledge, this observation is the first report so far that confirms diagnostic value and applicability of serum ADA activity to distinct HIV mono- and co-infection.

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